Docket No.: 077529.0112 (PATENT)

AMENDMENTS TO THE CLAIMS

This listing of the Claims replaces all prior versions, and listings, of the claims in the application:

- 1-39. (canceled)
- 40. (currently amended) A method of <u>for quantitating ex vivo</u> a <u>population of diagnosis or monitoring of infection with an intracellular pathogen in an individual-wherein peptide-specific immediate effector T cells present in vivo in a <u>subject</u> are enumerated, which method comprises:</u>
- (a) providing a fluid sample from said <u>subject individual</u> containing fresh T cells, which have not been cultured in vitro for a period of time <u>sufficient</u> to effect differentiation of precursor effector T cells to immediate effector T cells.
- (b) contacting said T cells in contact with a surface carrying an immobilized antibody to interferon-y.
- (bc) presenting to the <u>said</u> T cells a T cell-activating an activating amount of said peptide derived from the pathogen in the absence of any antigen presenting cells pre-cultured with said peptide,
- (e d) incubating the fluid sample said T cells under conditions to permit release of said interferon-γ but where the incubation time is not sufficient to effect differentiation of precursor effector T cells to immediate effector T cells, and
- (de) detecting released said interferon-y released in response to said peptide and bound to said immobilized antibody to enumerate said peptide specific effector T cells;

wherein the incubation is for a time to permit interferon γ release by only those T cells that have been pre-sensitized *in vivo* to the T cell-activating peptide and are capable of immediate effector

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function without the need to effect division/differentiation by *in vitro* culture in the presence of the T cell-activating peptide; whereby said infection is diagnosed or monitored.

- 41. (currently amended) The method as claimed in claim 40, wherein <u>said peptide is derived</u> from an the intracellular pathogen is selected from the group consisting of hepatitis B virus; hepatitis C virus, M. tuberculosis, P. falciparum, human immunodeficiency virus (HIV), and influenza virus.
- (currently amended) The method as claimed in claim 40 41, wherein said peptide is an ESAT-6 peptide of M. tuberculosis is presented to the T cells.
- (currently amended) The method as claimed in claim 40, wherein the <u>said</u> T cells are peripheral blood mononuclear cells.
- 44. (currently amended) The method as claimed in claim 40, wherein a <u>said</u> peptide <u>has</u> of 7-12 amino acid residues in length is <u>added to the T-cell-containing fluid</u>, which <u>and</u> is recognized by CD8+ T cells.
- 45. (currently amended) The method as claimed in claim 40, wherein the resulting fluid mixture is incubated said incubation is under non-sterile conditions.
- 46. (currently amended) The method as claimed in claim 40 41, wherein the <u>said</u> peptide is a pre-identified epitope from a protein of the <u>said</u> intracellular pathogen.
- 47. (currently amended) The method as claimed in claim 40, wherein <u>said</u> incubation is continued for a time of 4 to 24 hours.
- 48. (currently amended) The method as claimed in claim 40, wherein the T cells are taken from a patient said subject is known to be suffering, or to have suffered from, infection with the an intracellular pathogen.

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 (currently amended) The method as claimed in claim 41, wherein the <u>said</u> intracellular pathogen is HIV.

- 50. (currently amended) The method as claimed in claim 40, wherein the individual said subject has been immunized with a vaccine.
- 51. (currently amended) A method of diagnosis or monitoring of infection with *M. tuberculosis* in an individual wherein peptide for quantitating *ex vivo* a population of ESAT-6 peptide-specific immediate effector T cells present *in vivo* in a subject are enumerated, which method comprises:
- (a) providing a fluid sample comprising peripheral blood mononuclear cells from said individual subject containing fresh T cells, which have not been cultured in vitro for a period of time sufficient to effect differentiation of precursor effector T cells to immediate effector T cells.
- (b) contacting said T cells in contact with a surface carrying an immobilized antibody to interferon-y,
- (b c) presenting an ESAT-6 peptide of M-tubereulosis to said T cells an activating amount of said ESAT-6 peptide in the fluid sample in the absence of any antigen presenting cells pre-cultured with said ESAT-6 peptide,
- (e d) incubating the resulting fluid sample said T cells under conditions to permit release of said interferon-γ but where the incubation time is not sufficient to effect differentiation of precursor effector T cells to immediate effector T cells, and
- (d e) detecting released <u>said</u> interferon-γ released in response to <u>said ESAT-6</u> peptide and bound to said immobilized antibody to enumerate said peptide specific effector T cells;

wherein the incubation is for a time to permit interferon γ release by only those T cells that have been pre-sensitized *in vivo* to the ESAT-6 peptide and are capable of immediate effector function without the need to effect division/differentiation by *in-vitro* culture in the presence of the

ESAT-6 peptide; whereby said infection is diagnosed or monitored.

52. (currently amended) The method as claimed in claim 51, wherein a <u>said ESAT-6</u> peptide has of 7-12 amino acid residues in length is <u>added to the T-cell containing fluid sample</u>, which and is recognized by CD8+ T cells.

53. (currently amended) The method as claimed in claim 51, wherein the peptide containing fluid sample is incubated said incubation is under non-sterile conditions.

54. (currently amended) The method as claimed in claim 51, wherein the peripheral blood mononuclear cells are taken from a patient said subject is known to be suffering, or to have suffered from infection with M. tuberculosis.

55. - 58. (cancelled)

59. (currently amended) The method as claimed in claim 51, wherein the <u>said</u> incubation is for a time from 4 to 24 hours.

 (currently amended) The method as claimed in claim 40, wherein the <u>said</u> incubation is for a time from 6 to 16 hours.

61. (cancelled)

 (currently amended) The method as claimed in claim 51, wherein the <u>said</u> incubation is for a time from 6 to 16 hours

 (currently amended) The method as claimed in claim 41, wherein the <u>said</u> intracellular pathogen is M. tuberculosis.

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 (new) The method as claimed in claim 40, further comprising enumerating said peptidespecific immediate effector T cells.

- 65. (new) The method as claimed in claim 41, wherein said intracellular pathogen is selected from a group consisting of hepatitis B virus, hepatitis C virus, M. tuberculosis, P. falciparum, human immunodeficiency virus (HIV), and influenza virus.
- 66. (new) The method as claimed in claim 48, whereby said infection is monitored.
- 67. (new) The method as claimed in claim 50, whereby the induction or maintenance of said peptide-specific T cells following said immunization is monitored.
- (new) The method as claimed in claim 51, further comprising enumerating said ESAT-6
 peptide-specific immediate effector T cells.
- 69. (new) The method as claimed in claim 54, whereby said infection is monitored.